525 Rec'd PCT/PTO 11 OCT 2000

FORM PTO-1390 (Modified) (REV 11-2000) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER
NIDN-10370

U.S APPLICATION NO. (IF KNOWN, SEE 37 CFR
To be assigned 09/673168

INTERNATIONAL APPLICATION NO	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/GB99/01228	**April 22, 1999**	**April 22, 1998**

TITLE OF INVENTION
Ultrasound Contrast Agent

OIPE JCS9 OCT 11 2000 PATENT & TRADEMARK OFFICE

APPLICANT(S) FOR DO/EO/US
Skurtveit, et al.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☒ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☒ A copy of the International Search Report (PCT/ISA/210).
8. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
9. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
10. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
11. ☒ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).

Items 13 to 20 below concern document(s) or information included:

13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☒ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☒ Certificate of Mailing by Express Mail
20. ☒ Other items or information:

Return Postcard

Copy of the International Application as Published by the International Bureau

09673168 .1102.00

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U.S APPLICATION NO .(IF KNOWN, SEE 37 CFR	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
09/673168 To be assigned	PCT/GB99/01228	NIDN-10370

1. The following fees are submitted:.

	CALCULATIONS	PTO USE ONLY
BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :		
☐ Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2) paid to USPTO and International Search Report not prepared by the EPO or JPO **$970.00**		
☒ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but Internation Search Report prepared by the EPO or JPO **$840.00**		
☐ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO **$690.00**		
☐ International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) **$670.00**		
☐ International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) **$96.00**		
ENTER APPROPRIATE BASIC FEE AMOUNT =	**$840.00**	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).	**$0.00**	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	22 - 20 =	2	x $18.00	**$36.00**	
Independent claims	2 - 3 =	0	x $78.00	**$0.00**	
Multiple Dependent Claims **(check if applicable).**			☐	**$0.00**	
TOTAL OF ABOVE CALCULATIONS =				**$876.00**	
Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28) **(check if applicable).** ☐				**$0.00**	
SUBTOTAL =				**$876.00**	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)). +				**$0.00**	
TOTAL NATIONAL FEE =				**$876.00**	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).** ☐				**$0.00**	
TOTAL FEES ENCLOSED =				**$876.00**	
				Amount to be: refunded	$
				charged	$

☐ A check in the amount of to cover the above fees is enclosed.

☒ Please charge my Deposit Account No. **500-588** in the amount of **$876.00** to cover the above fees. A duplicate copy of this sheet is enclosed.

☒ The Commissioner is hereby authorized to charge any fees which may be required, or credit any overpayment to Deposit Account No. **500-588** A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Royal N. Ronning, Jr.
Amersham Pharmacia Biotech, Inc.
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[signature]
SIGNATURE

Royal N. Ronning, Jr.
NAME

32,529
REGISTRATION NUMBER

October 11, 2000
DATE

 NIDN-10370 #3

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of:	Skurtveit, et al.	Group Art Unit: To be assigned
Serial Number:	09/673,168	Examiner: To be assigned
Filing Date:	11 October 2000	
For:	Ultrasound Contrast Agent	

SECOND PRELIMINARY AMENDMENT

Honorable Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Applicants respectfully request the following amendment be considered to correct an error in the captioned application:

In the claims:

Please amend the following claim:

In Claim 1, line 1, please delete "presentation" and insert therein - - preparation - -.

Remarks

Claims 1-22 are pending in the captioned application, which application claims priority to PCT/GB99/01228. Applicants have amended a clerical error in Claim 1, wherein the word "presentation" instead of the correct word "preparation" was incorrectly published. Applicants respectfully assert that this amendment is fairly based on the specification (see page 3, lines 28-36) and respectfully request its entry. Applicant believes that the claims, as amended, are in allowable form, and earnestly solicits the allowance of Claims 1-22.

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Respectfully submitted,

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NIDN-10370

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: R. Skurtveit, et al.

Group Art Unit: To be assigned

Serial Number: To be assigned

Examiner: To be assigned

Filing Date: October 11, 2000

Title: Ultrasound Contrast Agent

FIRST PRELIMINARY AMENDMENT

Honorable Assistant Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

Applicants respectfully request the entry of the following preliminary amendment in connection with the prosecution of the captioned application, which claims priority to PCT Patent Application PCT/GB99/01228.

IN THE CLAIMS

In Claim 5, lines 1-2, please delete "any of the preceding claims" without prejudice and substitute--Claim 1--therefor.

In Claim 10, lines 1-2, please delete "any of the preceding claims" without prejudice and substitute--Claim 1--therefor.

In Claim 13, lines 1-2, please delete "any of the preceding claims" without prejudice and substitute--Claim 1--therefor.

In Claim 15, lines 1-2, please delete "any of claims 1-12" without prejudice and substitute --Claim 1--therefor.

In Claim 16, lines 1-2, please delete "any of claims 1-12" without prejudice and substitute --Claim 1--therefor.

In Claim 19, lines 1, please delete "or claim 18" without prejudice.

In Claim 21, lines 1-2, please delete "any of claims 1-12" without prejudice and substitute --Claim 1--therefor.

REMARKS

Claims 1-22 are pending in the captioned application, which application claims to PCT/GB99/01228.

Applicants have amended Claims 5, 10, 13, 15, 16, 19, and 21 to delete multiple dependencies. Applicants respectfully submit that the claims, as amended, are fairly based on the specification, and respectfully request their entry.

Applicants respectfully assert their Claims 1-22, are as amended, are allowable, and earnestly solicit their allowance.

Respectfully submitted,

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ULTRASOUND CONTRAST AGENT DISPERSIONS COMPRISING GAS AND DESTABILISING AGENT

This invention relates to ultrasound imaging, more particularly to novel contrast agent preparations and their use in ultrasound imaging, for example in visualising tissue perfusion.

It is well known that contrast agents comprising dispersions of microbubbles of gases are particularly efficient backscatterers of ultrasound by virtue of the low density and ease of compressibility of the microbubbles. Such microbubble dispersions, if appropriately stabilised, may permit highly effective ultrasound visualisation of, for example, the vascular system and tissue microvasculature, often at advantageously low doses.

The use of ultrasonography to measure blood perfusion (i.e. blood flow per unit of tissue mass) is of potential value in, for example, tumour detection, tumour tissue typically having different vascularity from healthy tissue, and studies of the myocardium, e.g. to detect myocardial infarctions. A problem with the application of existing ultrasound contrast agents to cardiac perfusion studies is that the information content of images obtained is degraded by attenuation caused by contrast agent present in the ventricles of the heart.

In our copending International Patent Publication No. WO-A-9817324, the contents of which are incorporated herein by reference, we have disclosed that ultrasonic visualisation of a subject, in particular of perfusion in the myocardium and other tissues, may be achieved and/or enhanced by means of gas-containing contrast agent preparations which promote controllable and temporary growth of the gas phase *in vivo* following administration. Such contrast agent preparations may be

used to promote controllable and temporary retention of the gas phase, for example in the form of microbubbles, in tissue microvasculature, thereby enhancing the concentration of gas in such tissue and accordingly enhancing its echogenicity, e.g. relative to the blood pool.

Such use of gas as a deposited perfusion tracer differs markedly from existing proposals regarding intravenously administrable microbubble ultrasound contrast agents. Thus it is generally thought necessary to avoid microbubble growth since, if uncontrolled, this may lead to potentially hazardous tissue embolisation. Accordingly it may be necessary to limit the dose administered and/or to use gas mixtures with compositions selected so as to minimise bubble growth *in vivo* by inhibiting inward diffusion of blood gases into the microbubbles (see e.g. WO-A-9503835 and WO-A-9516467).

In accordance with WO-A-9817324, on the other hand, a composition comprising a dispersed gas phase is coadministered with a composition comprising at least one substance which has or is capable of generating a gas or vapour pressure *in vivo* sufficient to promote controllable growth of the said dispersed gas phase through inward diffusion thereto of molecules of gas or vapour derived from said substance. This latter substance is referred to as a "diffusible component", although it will be appreciated that transport mechanisms other than diffusion may additionally or alternatively be involved.

The preparations may advantageously be employed in visualising tissue perfusion in a subject, the increase in size of the dispersed gas being utilised to effect enrichment or temporary retention of gas in the microvasculature of such tissue, thereby enhancing its echogenicity.

The dispersed gas content of such contrast agent

preparations will tend to be temporarily retained in tissue in concentrations proportional to the regional rate of tissue perfusion. Accordingly, when using ultrasound imaging modalities such as conventional or harmonic B-mode imaging where the display is derived directly from return signal intensities, images of such tissue may be interpreted as perfusion maps in which the displayed signal intensity is a function of local perfusion. This is in contrast to images obtained using free-flowing contrast agents, where the regional concentration of contrast agent and corresponding return signal intensity depend on the actual blood content rather than the rate of perfusion of local tissue.

The present invention is based on the finding that growth of a dispersed gas in order to bring about its temporary retention in tissue microvasculature may be effected by administering the gas dispersion in conjunction with one or more substances serving to destabilise the dispersion. By avoiding use of a diffusible component requiring transport through blood and/or the continuous phase(s) in which the gas dispersion and diffusible component are contained, as well as through any encapsulating or other stabilising membranes which surround them, the invention may allow greater control of growth of the dispersed gas and may also involve a higher proportion of gas-containing structures in the growth process.

Thus according to one aspect of the present invention there is provided a combined preparation for simultaneous, separate or sequential use as a contrast agent in ultrasound imaging, said preparation comprising:

i) an injectable aqueous gas dispersion, and

ii) an administrable substance or substances capable of destabilising said dispersed gas so as at least transiently to increase the size thereof.

According to a further aspect of the invention

there is provided a method of generating enhanced images of a human or non-human animal subject which comprises the steps of:

i) injecting a physiologically acceptable aqueous medium having gas dispersed therein into the vascular system of said subject;

ii) before, during or after injection of said aqueous medium administering to said subject a substance or substances capable of destabilising said dispersed gas so as at least transiently to increase the size thereof; and

iii) generating an ultrasound image of at least a part of said subject.

In general any biocompatible gas may be present in the gas dispersion used in accordance with the invention, the term "gas" as used herein including any substances (including mixtures) at least partially, e.g. substantially or completely, in gaseous or vapour form at the normal human body temperature of 37°C. Representative gases thus include air; nitrogen; oxygen; carbon dioxide; hydrogen; inert gases such as helium, argon, xenon or krypton; sulphur fluorides such as sulphur hexafluoride, disulphur decafluoride or trifluoromethylsulphur pentafluoride; selenium hexafluoride; optionally halogenated silanes such as methylsilane or dimethylsilane; low molecular weight hydrocarbons (e.g. containing up to 7 carbon atoms), for example alkanes such as methane, ethane, a propane, a butane or a pentane, cycloalkanes such as cyclopropane, cyclobutane or cyclopentane, alkenes such as ethylene, propene, propadiene or a butene, or alkynes such as acetylene or propyne; ethers such as dimethyl ether; ketones; esters; halogenated low molecular weight hydrocarbons (e.g. containing up to 7 carbon atoms); or mixtures of any of the foregoing. Advantageously at least some of the halogen atoms in halogenated gases are fluorine atoms; thus biocompatible halogenated

hydrocarbon gases may, for example, be selected from bromochlorodifluoromethane, chlorodifluoromethane, dichlorodifluoromethane, bromotrifluoromethane, chlorotrifluoromethane, chloropentafluoroethane, dichlorotetrafluoroethane, chlorotrifluoroethylene, fluoroethylene, ethylfluoride, 1,1-difluoroethane and perfluorocarbons. Representative perfluorocarbons include perfluoroalkanes such as perfluoromethane, perfluoroethane, perfluoropropanes or perfluorobutanes (e.g. perfluoro-n-butane, optionally in admixture with other isomers such as perfluoro-iso-butane); perfluoroalkenes such as perfluoropropene, perfluorobutenes (e.g. perfluorobut-2-ene), perfluorobutadiene, perfluoropentenes (e.g. perfluoropent-1-ene) or perfluoro-4-methylpent-2-ene; perfluoroalkynes such as perfluorobut-2-yne; and perfluorocycloalkanes such as perfluorocyclobutane, perfluoromethylcyclobutane, perfluorodimethylcyclobutanes, perfluorotrimethylcyclobutanes, perfluorocyclopentane, perfluoromethylcyclopentane, perfluorodimethylcyclopentanes, perfluorocyclohexane, perfluoromethylcyclohexane or perfluorocycloheptane. Other halogenated gases include methyl chloride, fluorinated (e.g. perfluorinated) ketones such as perfluoroacetone and fluorinated (e.g. perfluorinated) ethers such as perfluorodiethyl ether. The use of perfluorinated gases, for example sulphur hexafluoride and perfluorocarbons such as perfluoropropane, perfluorobutanes, perfluoropentanes and perfluorohexanes, may be particularly advantageous in view of the recognised high stability in the bloodstream of microbubbles containing such gases. Other gases with physicochemical characteristics which cause them to form highly stable microbubbles in the bloodstream may likewise be useful.

The dispersed gas may, for example, be present in the form of microbubbles at least partially encapsulated

or otherwise stabilised by gas-stabilising material. This stabilising material may, for example, comprise an initially coalescence-resisting surface membrane (for example gelatin, e.g. as described in WO-A-8002365), a filmogenic protein (for example an albumin such as human serum albumin, e.g. as described in US-A-4718433, US-A-4774958, US-A-4844882, EP-A-0359246, WO-A-9112823, WO-A-9205806, WO-A-9217213, WO-A-9406477 or WO-A-9501187), a polymer material (for example a synthetic biodegradable polymer as described in EP-A-0398935, an elastic interfacial synthetic polymer membrane as described in EP-A-0458745, a microparticulate biodegradable polyaldehyde as described in EP-A-0441468, a microparticulate N-dicarboxylic acid derivative of a polyamino acid - polycyclic imide as described in EP-A-0458079, or a biodegradable polymer as described in WO-A-9317718 or WO-A-9607434), a non-polymeric and non-polymerisable wall-forming material (for example as described in WO-A-9521631), or a surfactant (for example a polyoxyethylene-polyoxypropylene block copolymer surfactant such as a Pluronic, a polymer surfactant as described in WO-A-9506518, or a film-forming surfactant such as a phospholipid, e.g. as described in WO-A-9211873, WO-A-9217212, WO-A-9222247, WO-A-9428780, WO-A-9503835 or WO-A-9729783).

Where phospholipid-containing gas dispersions are employed in accordance with the invention, e.g. in the form of phospholipid-stabilised gas microbubbles, representative examples of useful phospholipids include lecithins (i.e. phosphatidylcholines), for example natural lecithins such as egg yolk lecithin or soya bean lecithin, semisynthetic (e.g. partially or fully hydrogenated) lecithins and synthetic lecithins such as dimyristoylphosphatidylcholine, dipalmitoylphosphatidylcholine or distearoylphosphatidylcholine; phosphatidic acids; phosphatidylethanolamines; phosphatidylserines;

phosphatidylglycerols; phosphatidylinositols; cardiolipins; sphingomyelins; fluorinated analogues of any of the foregoing; mixtures of any of the foregoing and mixtures with other lipids such as cholesterol. The use of phospholipids predominantly (e.g. at least 75%) comprising molecules individually bearing net overall charge, for example as described in WO-A-9729783, may be particularly advantageous. Representative negatively charged phospholipids include naturally occurring (e.g. soya bean or egg yolk derived), semisynthetic (e.g. partially or fully hydrogenated) and synthetic phosphatidylserines, phosphatidylglycerols, phosphatidylinositols, phosphatidic acids and/or cardiolipins. Representative positively charged phospholipids include esters of phosphatidic acids such as dipalmitoylphosphatidic acid or distearoyl-phosphatidic acid with aminoalcohols such as hydroxy-ethylenediamine.

The disclosures of all of the above-described documents relating to gas-containing contrast agent formulations are incorporated herein by reference.

Gas microbubbles preferably have an initial average size not exceeding 10 μm (e.g. of 7 μm or less) in order to permit their free passage through the pulmonary system following administration, e.g. by intravenous injection. However, larger microbubbles may be employed where, for example, these contain a mixture of one or more relatively blood-soluble or otherwise diffusible gases such as air, oxygen, nitrogen or carbon dioxide with one or more substantially insoluble and non-diffusible gases such as perfluorocarbons. Outward diffusion of the soluble/diffusible gas content following administration will cause such microbubbles rapidly to shrink to a size which will be determined by the amount of insoluble/non-diffusible gas present and which may be selected to permit passage of the resulting microbubbles through the lung capillaries of the

pulmonary system.

The destabilising substance(s) (hereinafter referred to as "the destabiliser") employed to induce at least transient growth of the dispersed gas phase may, for example, operate by mechanisms involving (i) flocculation, aggregation or agglomeration whereby dispersed gas microbubbles assemble together to form clusters; (ii) coalescence or fusion of the dispersed gas moieties whereby two or more such moieties merge to create a larger moiety; or (iii) Ostwald ripening of dispersed gas moieties, where larger microbubbles grow at the expense of smaller ones, the driving force being the higher pressure in small microbubbles compared to large microbubbles as a result of differences in surface tension in accordance with Laplace's law. It will be appreciated that two or more mechanisms may operate simultaneously.

Flocculation, aggregation or agglomeration may be achieved by making the interbubble repulsive forces weaker than the interbubble attractive forces. The size of the resulting clusters may depend on factors such as the initial size and concentration of the microbubbles, the hydrodynamic behaviour of the surrounding blood stream, the ambient temperature and the net attractive force between the microbubbles.

Where the dispersed gas is stabilised by charge interactions, for example by interactions between charged surface groups in stabilising membranes of materials such as charged phospholipids, addition of charged species such as electrolytes will supply counter-ions which will screen the charged surface groups and reduce interbubble repulsive forces. The use of di- and multi-valent counter-ions is preferred; thus, for example, salts, e.g. inorganic salts such as calcium chloride or magnesium chloride may be used to destabilise negatively charged membranes.

Aggregation may also be induced by modifying the

properties of the surrounding water phase, for example by addition of solvents or of substances which change the electrical permeability of the medium so as to destabilise charge-stabilised gas dispersions. Representative examples of such substances include water-miscible liquids or solids, e.g. aliphatic alcohols such as ethanol, isopropanol, ethylene glycol, propylene glycol, glycerol and sorbitol; aliphatic aldehydes and ketones such as acetaldehyde and acetone; aliphatic esters such as methyl acetate, propyl formate and ethyl acetate; aliphatic ethers such as methyl propyl ether and di-isopropyl ether; aliphatic amides such as N,N-dimethylformamide and N,N-dimethylacetamide; aliphatic nitriles such as acetonitrile; carbohydrates such as glucose and sucrose; polyethylene glycol; polypropylene glycol etc. Aliphatic compounds as described herein typically contain up to 6 carbon atoms.

Other representative destabilisers include components capable of bridging dispersed gas microbubbles. Examples of such components include polymers, e.g. polysaccharides such as dextran and starches; polyaminoacids such as polylysine and proteins such as gelatin and albumin; polyethers such as polyethylene glycol, polypropylene glycol and polyoxyethylene-polyoxypropylene block copolymers such as Pluronics; polyvinyl pyrrolidone etc.

Aggregation may also be induced by adding components which enter and modify the membrane, thereby altering the forces between different microbubbles. Examples of such compounds include fatty alcohols, fatty acids and fatty amines; various surfactants; steroids such as cholesterol etc.

Where the gas-stabilising material contains acid or base groups on its surface, destabilisation may be effected by addition of an appropriate acidic or basic destabiliser in order to change the pH.

Aggregation may be a reversible or irreversible

process, depending on the magnitude of the forces between the dispersed gas moieties. By optimising these forces the aggregates may be made loose enough to be easily disintegrated a short time after their temporary retention in tissue microvasculature, thus ensuring that the imaging procedure has a high safety profile. Such disintegration or redispersion of the aggregates may if desired be induced or enhanced by subsequent administration of an appropriate substance. For instance, a complexing agent such as ethylenediamine tetraacetic acid may remove or neutralise the effect of divalent or polyvalent ions used to destabilise charged microbubbles. A buffer, acid or base may be used to remove the effect of pH-induced aggregation/ destabilistation. Solvents, e.g. alcohols such as ethanol, isopropanol, ethylene glycol, propylene glycol and glycerol; aldehydes and ketones such as acetaldehyde and acetone; ethers such as methyl propyl ether and di-isopropyl ether; aliphatic amides such as N,N-dimethylformamide and N,N-dimethylacetamide; or aliphatic nitriles such as acetonitrile, may for example be added to change the bridging behaviour of a polymeric flocculant by altering the polymer's ability to coil in the solvent. Such substances therefore re-establish the repulsive forces between the dispersed gas moieties.

Destabilisers which operate by inducing coalescence or fusion of the dispersed gas moieties include substances which modify the properties of gas-stabilising membrane material so that membrane rupture between coalescing gas moieties is enhanced. Representative destabilisers of this type include surfactants, co-surfactants, solvents and other substances which penetrate or otherwise modify the properties of the stabilising membrane. Chemical modification of the stabilising membrane, e.g. by addition of electrolytes or by pH change, or chemical reaction within the stabilising membrane may likewise be

used to induce flocculation.

Growth of dispersed gas moieties by Ostwald ripening may be initiated by increasing the solubility of the gas in the surrounding aqueous phase, for example by adding a solvent or other appropriate component, e.g. a hydrotrope such as sodium benzoate or acetic acid, or a surfactant capable of solubilising the gas within micelles. Representative examples of solvents which may increase the solubility of the gas in the surrounding aqueous phase include aliphatic ethers such as ethyl methyl ether and methyl propyl ether; aliphatic esters such as methyl acetate, methyl formate and ethyl formate; aliphatic ketones such as acetone; aliphatic amides such as N,N-dimethylformamide and N,N-dimethylacetamide; and aliphatic nitriles such as acetonitrile. Representative examples of surfactants which may increase the solubility of the gas in the surrounding aqueous phase are those forming micelles in water, e.g. alkyl carboxylates, alkyl sulphonates, alkyl sulphates, dialkyl sulphosuccinates, alkylpyridinium salts, alkylammonium salts, alkyl polyethylene glycol ethers, alkyl polyethylene glycol esters and sorbitol fatty acid esters; alkyl groups in such surfactants may, for example, contain 5-30 carbon atoms.

Ostwald ripening kinetics may also be enhanced by increasing the interfacial tension about the dispersed gas, for example by modifying the properties of stabilising membranes, e.g. through solvent addition, salt addition, pH change or addition of surfactants/ co-surfactants which provide higher surface tension.

As noted above, the destabiliser may be administered before, during or after injection of the gas dispersion; the order of administration and timing between them when they are separate may be varied to permit control over the time, rate and thus location of temporary retention of the dispersed gas.

In many instances the destabiliser will be

administered by injection, e.g. intravenously, although intramuscular or subcutaneous injections may be useful in order to localise the effect of the destabiliser. Volatile destabilisers, e.g. solvents such as ethanol, may, however, also be administered by inhalation; this may be of advantage in avoiding unwanted growth of the dispersed gas in the lung capillaries.

Imaging modalities which may be used in accordance with the invention include two- and three-dimensional imaging techniques such as B-mode imaging (for example using the time-varying amplitude of the signal envelope generated from the fundamental frequency of the emitted ultrasound pulse, from sub-harmonics or higher harmonics thereof or from sum or difference frequencies derived from the emitted pulse and such harmonics, images generated from the fundamental frequency or the second harmonic thereof being preferred), colour Doppler imaging, Doppler amplitude imaging and combinations of these last two techniques with any of the other modalities described above. To reduce the effects of movement, successive images of tissues such as the heart or kidney may be collected with the aid of suitable synchronisation techniques (e.g. gating to the ECG or respiratory movement of the subject). Measurement of changes in resonance frequency or frequency absorption which accompany growth of the dispersed gas may also usefully be made to detect the contrast agent.

It will be appreciated that the dispersed gas content of combined contrast agent preparations according to the invention will, following destabilisation, tend to be temporarily retained in tissue in concentrations proportional to the regional rate of tissue perfusion. Accordingly, when using ultrasound imaging modalities such as conventional or harmonic B-mode imaging where the display is derived directly from return signal intensities, images of such tissue may be interpreted as perfusion maps in which the

displayed signal intensity is a function of local perfusion. This is in contrast to images obtained using free-flowing contrast agents, where the regional concentration of contrast agent and corresponding return signal intensity depend on the actual blood content rather than the rate of perfusion of local tissue.

In cardiac studies, where perfusion maps are derived from return signal intensities in accordance with this embodiment of the invention, it may be advantageous to subject a patient to physical or pharmacological stress in order to enhance the distinction, and thus the difference in image intensities, between normally perfused myocardium and any myocardial regions supplied by stenotic arteries. As is known from radionuclide cardiac imaging, such stress induces vasodilatation and increased blood flow in healthy myocardial tissue, whereas blood flow in underperfused tissue supplied by a stenotic artery is substantially unchanged since the capacity for arteriolar vasodilatation is already exhausted by inherent autoregulation seeking to increase the restricted blood flow.

The application of stress as physical exercise or pharmacologically by administration of adrenergic agonists may cause discomfort such as chest pains in patient groups potentially suffering from heart disease, and it is therefore preferable to enhance the perfusion of healthy tissue by administration of a vasodilating drug, for example selected from adenosine, dipyridamole, nitroglycerine, isosorbide mononitrate, prazosin, doxazosin, dihydralazine, hydralazine, sodium nitroprusside, pentoxyphylline, amelodipine, felodipine, isradipine, nifedipine, nimodipine, verapamil, diltiazem and nitrous oxide. In the case of adenosine this may lead to in excess of fourfold increases in coronary blood flow in healthy myocardial tissue, greatly increasing the uptake and temporary retention of

contrast agents in accordance with the invention and thus significantly increasing the difference in return signal intensities between normal and hypoperfused myocardial tissue. Because an essentially physical entrapment process is involved, retention of contrast agents according to the invention is highly efficient; this may be compared to the uptake of radionucleide tracers such as thallium 201 and technetium sestamibi, which is limited by low contact time between tracer and tissue and so may require maintenance of vasodilatation for the whole period of blood pool distribution for the tracer (e.g. 4-6 minutes for thallium scintigraphy) to ensure optimum effect. The contrast agents of the invention, on the other hand, do not suffer such diffusion or transport limitations, and since their retention in myocardial tissue may also rapidly be terminated by the methods described above, the period of vasodilatation needed to achieve cardiac perfusion imaging in accordance with this embodiment of the invention may be very short, for example less than one minute. This will reduce the duration of any possible discomfort caused to patients by administration of vasodilator drugs.

In view of the fact that the required vasodilatation need only be short lasting, adenosine is a particularly useful vasodilating drug, being both an endogenous substance and having a very short-lasting action as evidenced by a blood pool half-life of only 2 seconds. Vasodilatation will accordingly be most intense in the heart, since the drug will tend to reach more distal tissues in less than pharmacologically active concentrations. It will be appreciated that because of this short half-life, repeated injection or infusion of adenosine may be necessary during cardiac imaging in accordance with this embodiment of the invention; by way of example, an initial administration of 150 μg/kg of adenosine may be made substantially

simultaneously with administration of the contrast agent composition, followed 10 seconds later by slow injection of a further 150 μg/kg of adenosine, e.g. over a period of 20 seconds.

Contrast agent preparations in accordance with the invention may advantageously be employed as delivery agents for bioactive moieties such as therapeutic drugs (i.e. agents having a beneficial effect on a specific disease in a living human or non-human animal), particularly to targeted sites. Thus, for example, therapeutic compounds may be present in the dispersed gas or may be linked to part of the stabilising material, e.g. through covalent or ionic bonds, if desired through a spacer arm.

The controllable growth properties of the dispersed gas may be utilised to bring about its temporary retention in the microvasculature of a target region of interest, and thus retention of the gas and associated therapeutic compound in a target structure is particularly advantageous.

The therapeutic compound, which may if desired be coupled to a site-specific vector having affinity for specific cells, structures or pathological sites, may be released as a result of, for example, stretching or fracture of the gas-stabilising material caused by growth of the dispersed gas, solubilisation of the stabilising material, or disintegration of gas-microbubbles or gas-containing microparticles. Where a therapeutic agent is chemically linked to the gas-stabilising material, the linkage or any spacer arm associated therewith may advantageously contain one or more labile groups which are cleavable to release the agent. Representative cleavable groups include amide, imide, imine, ester, anhydride, acetal, carbamate, carbonate, carbonate ester and disulphide groups which are biodegradable *in vivo*, e.g. as a result or hydrolytic and/or enzymatic action.

Representative and non-limiting examples of drugs useful in accordance with this embodiment of the invention include antineoplastic agents such as vincristine, vinblastine, vindesine, busulfan, chlorambucil, spiroplatin, cisplatin, carboplatin, methotrexate, adriamycin, mitomycin, bleomycin, cytosine arabinoside, arabinosyl adenine, mercaptopurine, mitotane, procarbazine, dactinomycin (antinomycin D), daunorubicin, doxorubicin hydrochloride, taxol, plicamycin, aminoglutethimide, estramustine, flutamide, leuprolide, megestrol acetate, tamoxifen, testolactone, trilostane, amsacrine (m-AMSA), asparaginase (L-asparaginase), etoposide, interferon a-2a and 2b, blood products such as hematoporphyrins or derivatives of the foregoing; biological response modifiers such as muramylpeptides; antifungal agents such as ketoconazole, nystatin, griseofulvin, flucytosine, miconazole or amphotericin B; hormones or hormone analogues such as growth hormone, melanocyte stimulating hormone, estradiol, beclomethasone dipropionate, betamethasone, cortisone acetate, dexamethasone, flunisolide, hydrocortisone, methylprednisolone, paramethasone acetate, prednisolone, prednisone, triamcinolone or fludrocortisone acetate; vitamins such as cyanocobalamin or retinoids; enzymes such as alkaline phosphatase or manganese superoxide dismutase; antiallergic agents such as amelexanox; anticoagulation agents such as warfarin, phenprocoumon or heparin; antithrombotic agents; circulatory drugs such as propranolol; metabolic potentiators such as glutathione; antituberculars such as p-aminosalicylic acid, isoniazid, capreomycin sulfate, cyclosexine, ethambutol, ethionamide, pyrazinamide, rifampin or streptomycin sulphate; antivirals such as acyclovir, amantadine, azidothymidine, ribavirin or vidarabine; blood vessel dilating agents such as diltiazem, nifedipine, verapamil, erythritol tetranitrate, isosorbide

dinitrate, nitroglycerin or pentaerythritol tetranitrate; antibiotics such as dapsone, chloramphenicol, neomycin, cefaclor, cefadroxil, cephalexin, cephradine, erythromycin, clindamycin, lincomycin, amoxicillin, ampicillin, bacampicillin, carbenicillin, dicloxacillin, cyclacillin, picloxacillin, hetacillin, methicillin, nafcillin, penicillin or tetracycline; antiinflammatories such as diflunisal, ibuprofen, indomethacin, meclefenamate, mefenamic acid, naproxen, phenylbutazone, piroxicam, tolmetin, aspirin or salicylates; antiprotozoans such as chloroquine, metronidazole, quinine or meglumine antimonate; antirheumatics such as penicillamine; narcotics such as paregoric; opiates such as codeine, morphine or opium; cardiac glycosides such as deslaneside, digitoxin, digoxin, digitalin or digitalis; neuromuscular blockers such as atracurium mesylate, gallamine triethiodide, hexafluorenium bromide, metocurine iodide, pancuronium bromide, succinylcholine chloride, tubocurarine chloride or vecuronium bromide; sedatives such as amobarbital, amobarbital sodium, apropbarbital, butabarbital sodium, chloral hydrate, ethchlorvynol, ethinamate, flurazepam hydrochloride, glutethimide, methotrimeprazine hydrochloride, methyprylon, midazolam hydrochloride, paraldehyde, pentobarbital, secobarbital sodium, talbutal, temazepam or triazolam; local anaesthetics such as bupivacaine, chloroprocaine, etidocaine, lidocaine, mepivacaine, procaine or tetracaine; general anaesthetics such as droperidol, etomidate, fentanyl citrate with droperidol, ketamine hydrochloride, methohexital sodium or thiopental and pharmaceutically acceptable salts (e.g. acid addition salts such as the hydrochloride or hydrobromide or base salts such as sodium, calcium or magnesium salts) or derivatives (e.g. acetates) thereof; and radiochemicals, e.g. comprising beta-emitters. Of particular importance are antithrombotic agents such as

vitamin K antagonists, heparin and agents with heparin-like activity such as antithrombin III, dalteparin and enoxaparin; blood platelet aggregation inhibitors such as ticlopidine, aspirin, dipyridamole, iloprost and abciximab; and thrombolytic enzymes such as streptokinase and plasminogen activator. Other examples of therapeutics include genetic material such as nucleic acids, RNA, and DNA of natural or synthetic origin, including recombinant RNA and DNA. DNA encoding certain proteins may be used in the treatment of many different types of diseases. For example, tumour necrosis factor or interleukin-2 may be provided to treat advanced cancers; thymidine kinase may be provided to treat ovarian cancer or brain tumors; interleukin-2 may be provided to treat neuroblastoma, malignant melanoma or kidney cancer; and interleukin-4 may be provided to treat cancer.

Contrast agent preparations in accordance with the invention may be used as vehicles for contrast-enhancing moieties for imaging modalities other than ultrasound, for example X-ray, light imaging, magnetic resonance and, more preferably, scintigraphic imaging agents. Controlled growth of the dispersed gas phase may be used to position such agents in areas of interest within the bodies of subjects, which may then be imaged.

Contrast agent preparations in accordance with the invention may also be used as vehicles for therapeutically active substances which do not necessarily require release from the preparation in order to exhibit their therapeutic effect. Such preparations may, for example, incorporate radioactive atoms or ions such as beta-emitters which exhibit a localised radiation-emitting effect following growth of the dispersed gas phase and temporary retention of the agent at a target site. It will be appreciated that such agents should preferably be designed so that subsequent shrinkage and cessation of retention of the

dispersed gas does not occur until the desired therapeutic radiation dosage has been administered.

Contrast agent preparations in accordance with the invention may additionally exhibit therapeutic properties in their own right. Thus, for example, the dispersed gas may be targeted to capillaries leading to tumours and may act as cell toxic agents by blocking such capillaries. Concentrations of dispersed gas in capillaries may also enhance absorption of ultrasonic energy in hyperthermic therapy; this may be used in, for example, treatment of liver tumours. Irradiation with a relatively high energy (e.g. 5 W) focused ultrasound beam, e.g. at 1.5 MHz, may be appropriate in such applications.

The following non-limitative Examples serve to illustrate the invention.

Preparation 1

Phosphatidylserine-stabilised perfluorobutane microbubble dispersion

Hydrogenated phosphatidylserine (5 mg/ml in a 1% w/w solution of propylene glycol in purified water) and perfluorobutane gas were homogenised in-line at 7800 rpm and *ca.* 40°C to yield a creamy-white microbubble dispersion. The dispersion was fractionated to substantially remove undersized microbubbles (<2 μm) and the volume of the dispersion was adjusted to the desired microbubble concentration by adding aqueous sucrose to give a sucrose concentration of 92 mg/ml. 2 ml portions of the resulting dispersion were filled into 10 ml flat-bottomed vials specially designed for lyophilisation, and the contents were lyophilised to give a white porous cake. The lyophilisation chamber was then filled with perfluorobutane and the vials were sealed. Prior to use, water was added to a vial and the contents were gently hand-shaken for several seconds to give a perfluorobutane microbubble dispersion.

Preparation 2

Phosphatidylserine-stabilised perfluorobutane microbubble dispersion

Hydrogenated egg phosphatidylserine (50 mg) was dispersed in water (9.75 ml, reversed osmosis quality) containing propylene glycol (0.25 ml) by heating to 80°C for about 2 minutes, followed by cooling to room temperature. 1 ml portions of the dispersion were transferred into ten 2 ml chromatography vials. The headspaces were flushed with perfluorobutane gas and the vials were capped and shaken on an Espe CapMix® shaker for 45 seconds, yielding samples of a milky white

microbubble dispersion. The microbubble dispersions were collected in two 7 ml vials which were capped and placed on a roller table for two days. Later the samples were stored standing at room temperature until use.

Preparation 3

Sodium dodecyl sulphate solution

Sodium dodecyl sulphate (2.09 g) was added to water (7 ml, reversed osmosis quality) at room temperature to obtain a clear solution.

Preparation 4

Brij 99 solution

Brij 99 (2.84 g) was added to water (9.5 ml, reversed osmosis quality) and placed on a roller table overnight. A clear, slightly viscous solution was obtained.

Preparation 5

Sodium dodecyl sulphate/n-butanol/water microemulsion

Sodium dodecyl sulphate (12.5810 g), n-butanol (27.0813 g) and water (17.07 g, reversed osmosis quality) were mixed and diluted further with water to a water content of 30% w/w, yielding a clear, transparent microemulsion.

EXAMPLE 1

Microbubble aggregation by calcium chloride

One drop of the microbubble dispersion prepared in Preparation 1 was placed on an object glass for

microscopy investigation. The sample was covered with a cover glass and placed under a microscope. Droplets of a 50 mg/ml calcium chloride solution in water were added to the edge of the cover glass so that the solution penetrated into the microbubble dispersion. The behaviour of the microbubble dispersion as the calcium chloride solution front moved was recorded on a video tape. Microbubble aggregates with larger dimensions than the initial microbubbles were observed to form, demonstrating that microbubbles with potential for retention in capillary systems were generated.

EXAMPLE 2

Microbubble aggregation by sodium dodecyl sulphate

One drop of the microbubble dispersion prepared in Preparation 2 was placed on an object glass and covered by a cover glass. The sample was placed under a Zeiss Axioskop optical microscope equipped with a 10 x objective and a camera connected to a computer and an image analysis system. A droplet of the sodium dodecyl sulphate solution from Preparation 3 was placed on the edge of the coverglass, so that the solution penetrated under the coverglass and into the microbubble dispersion. The behaviour of the microbubble dispersion as the sodium dodecyl sulphate solution front moved was recorded, showing formation of microbubble aggregates with larger dimensions than the initial microbubbles, demonstrating the formation of microbubbles with potential for retention in capillary systems.

EXAMPLE 3

Microbubble aggregation by Brij 99

The procedure described in Example 2 was repeated using

the same microbubble dispersion from Preparation 2 but adding the Brij 99 surfactant solution from Preparation 4 to the edge of the coverglass. The experiment showed formation of microbubble aggregates with larger dimensions than the initial microbubbles, demonstrating the formation of microbubbles with potential for retention in capillary systems.

EXAMPLE 4

Microbubble growth by ethanol

The procedure described in Example 2 was repeated using the same microbubble dispersions from Preparation 2 but adding pure ethanol to the edge of the coverglass. The experiment showed growth of microbubbles resulting in microbubbles of larger size than the initial microbubbles, thereby demonstrating the formation of microbubbles with potential for retention in capillary systems.

EXAMPLE 5

Microbubble growth by isopropanol

The procedure described in Example 2 was repeated using the same microbubble dispersion from Preparation 2 but adding pure isopropanol to the edge of the coverglass. The experiment showed growth of microbubbles resulting in microbubbles of larger size than the initial microbubbles, thereby demonstrating the formation of microbubbles with potential for retention in capillary systems.

EXAMPLE 6

Microbubble growth by acetone

The procedure described in Example 2 was repeated using the same microbubble dispersion from Preparation 2 but adding pure acetone to the edge of the coverglass. The experiment showed growth of microbubbles resulting in microbubbles of larger size than the initial microbubbles, thereby demonstrating the formation of microbubbles with potential for retention in capillary systems.

EXAMPLE 7

Microbubble aggregation and growth by a sodium dodecyl sulphate/n-butanol/water microemulsion

One droplet of the microbubble dispersion from Preparation 2, diluted three times with water was placed on an object glass and covered by a coverglass. The sample was placed under a Zeiss Axioskop optical microscope equipped with a 40 x objective and a camera connected to a computer and an image analysis system. A droplet of the sodium dodecyl sulphate/n-butanol/water microemulsion from Preparation 5 was placed on the edge of the coverglass, so that the microemulsion penetrated under the coverglass and into the microbubble dispersion. The behaviour of the microbubble dispersion as the microemulsion front advanced was recorded, showing formation of microbubble aggregates with larger dimensions than the initial microbubbles. A short time afterwards, the microbubbles in the aggregates fused or coalesced to give larger microbubbles, demonstrating the formation of microbubbles with potential for retention in capillary systems.

Claims:

1. A combined presentation for simultaneous, separate or sequential use as an ultrasound contrast agent, said preparation comprising:
   - i) an injectable aqueous gas dispersion; and
   - ii) a separately administrable substance or substances capable of destabilising said dispersed gas so as at least transiently to increase the size thereof.

2. A combined preparation as claimed in claim 1 wherein the dispersed gas comprises air, nitrogen, oxygen, carbon dioxide, hydrogen, an inert gas, a sulphur fluoride, selenium hexafluoride, an optionally halogenated silane, an optionally halogenated low molecular weight hydrocarbon, a ketone, an ester or a mixture of any of the foregoing.

3. A combined preparation as claimed in claim 2 wherein the dispersed gas comprises sulphur hexafluoride or a perfluorocarbon.

4. A combined preparation as claimed in claim 3 wherein said perfluorocarbon is perfluoropropane, perfluorobutane or perfluoropentane.

5. A combined preparation as claimed in any of the preceding claims wherein the dispersed gas is stabilised by an initially coalescence-resisting surface membrane, a filmogenic protein, a polymer material, a non-polymeric and non-polymerisable wall-forming material or a surfactant.

6. A combined preparation as claimed in claim 5 wherein said surfactant comprises at least one phospholipid.

7. A combined preparation as claimed in claim 6 wherein at least 75% of said surfactant comprises phospholipid molecules individually bearing net overall charge.

8. A combined preparation as claimed in claim 7 wherein said charged phospholipid molecules are selected from phosphatidylserine, phosphatidylglycerol, phosphatidylinositol, phosphatidic acid and cardiolipin molecules.

10. A combined preparation as claimed in any of the preceding claims comprising one or more destabilising substances which induce growth of the dispersed gas by flocculation, aggregation, agglomeration, coalescence, fusion or Ostwald ripening.

11. A combined preparation as claimed in claim 10 comprising one or more destabilising substances selected from inorganic salts, aliphatic alcohols, aliphatic aldehydes, aliphatic ketones, aliphatic esters, aliphatic ethers, aliphatic amides, aliphatic nitriles, carbohydrates, polyethers, polysaccarides, polyaminoacids, polyvinylpyrrolidone, fatty alcohols, fatty acids, fatty amines, surfactants, steroids, acids, bases and hydrotropes.

12. A combined preparation as claimed in claim 11 comprising one or more destabilising substances selected from calcium chloride, magnesium chloride, ethanol, isopropanol, ethylene glycol, propylene glycol, glycerol, sorbitol, acetaldehyde, acetone, methyl formate, methyl acetate, propyl formate, ethyl acetate, ethyl methyl ether, methyl propyl ether, di-isopropyl ether, N,N-dimethylformamide, N,N-dimethylacetamide, acetonitrile, glucose, sucrose, polyethylene glycol, polypropylene glycol, polyoxyethylene-polyoxypropylene

block copolymers, dextran, starches, polylysine, gelatin, cholesterol, and surface active alkyl carboxylates, alkyl sulphonates, alkyl sulphates, dialkyl sulphosuccinates, alkyl pyridinium salts, alkylammonium salts, alkyl polyethylene glycol ethers, alkyl polyethylene glycol esters and sorbitol fatty acid esters.

13. A combined preparation as claimed in any of the preceding claims which further includes a vasodilator drug.

14. A combined preparation as claimed in claim 13 wherein said vasodilator drug is adenosine.

15. A combined preparation as claimed in any of claims 1 to 12 which further includes a therapeutic drug.

16. A combined preparation as claimed in any of claims 1 to 12 which further includes contrast-enhancing moieties for an imaging modality other than ultrasound.

17. A method of generating enhanced images of a human or non-human animal subject which comprises the steps of:

i) injecting a physiologically acceptable aqueous medium having gas dispersed therein into the vascular system of said subject;

ii) before, during or after injection of said aqueous medium administering to said subject a substance or substances capable of destabilising said dispersed gas so as at least transiently to increase the size thereof; and

iii) generating an ultrasound image of at least a part of said subject.

18. A method as claimed in claim 17 wherein

destabilising substance is administered subcutaneously, intramuscularly, intravenously or by inhalation.

19. A method as claimed in claim 17 or claim 18 wherein a vasodilator drug is coadministered to the subject.

20. A method as claimed in claim 19 wherein said vasodilator drug is adenosine.

21. Use of a contrast agent as claimed in any of claims 1 to 12 in ultrasound therapy.

22. Use as claimed in claim 21 wherein said therapy involves cell killing or blocking of blood flow to a site of interest.

Please type a plus sign (+) inside this box → [+]

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DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION (37 CFR 1.63)

☐ Declaration Submitted with Initial Filing OR ☒ Declaration Submitted after Initial Filing (surcharge (37 CFR 1.16 (e)) required)

Attorney Docket Number	NIDN-10370
First Named Inventor	Skurtveit
COMPLETE IF KNOWN	
Application Number	09/673,168
Filing Date	11-Oct-2000
Group Art Unit	To be assigned
Examiner Name	To be assigned

As a below named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

Ultrasound Contrast Agent

(Title of the Invention)

the specification of which

☐ is attached hereto

OR

☒ was filed on (MM/DD/YYYY) 10/11/2000 as United States Application Number or PCT International Application Number 09/673,168 and was amended on (MM/DD/YYYY) ____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Priority Not Claimed	Certified Copy Attached? YES	NO
9808582.2	GB	04/22/1998	☐	☐	☒
			☐	☐	☐
			☐	☐	☐
			☐	☐	☐

☐ Additional foreign application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto:

I hereby claim the benefit under 35 U.S.C. 119(e) of any United States provisional application(s) listed below.

Application Number(s)	Filing Date (MM/DD/YYYY)
60/084,881	05/08/1998

☐ Additional provisional application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto.

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DECLARATION — Utility or Design Patent Application

I hereby claim the benefit under 35 U.S.C. 120 of any United States application(s), or 365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. 112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

U.S. Parent Application or PCT Parent Number	Parent Filing Date (MM/DD/YYYY)	Parent Patent Number (if applicable)
PCT/GB99/01228 which is a CIP of US 60/084,881 filed 05/08/1998	04/22/1999	

☐ Additional U.S. or PCT international application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto.

As a named inventor, I hereby appoint the following registered practitioner(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith: ☒ Customer Number 22840 → Place Customer Number Bar Code Label here: 22840
OR
☐ Registered practitioner(s) name/registration number listed below

PATENT TRADEMARK OFFICE

Name	Registration Number	Name	Registration Number

☐ Additional registered practitioner(s) named on supplemental Registered Practitioner Information sheet PTO/SB/02C attached hereto.

Direct all correspondence to: ☒ Customer Number or Bar Code Label 22840 OR ☐ Correspondence address below

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City		State		ZIP	
Country		Telephone		Fax	

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Name of Sole or First Inventor: ☐ A petition has been filed for this unsigned inventor

1-00

Given Name (first and middle [if any])	Family Name or Surname
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☒ Additional inventors are being named on the 1 supplemental Additional Inventor(s) sheet(s) PTO/SB/02A attached hereto

09/674592

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DECLARATION

ADDITIONAL INVENTOR(S)
Supplemental Sheet
Page 1 of 1

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Post Office Address							
Post Office Address							
City		State		ZIP		Country	